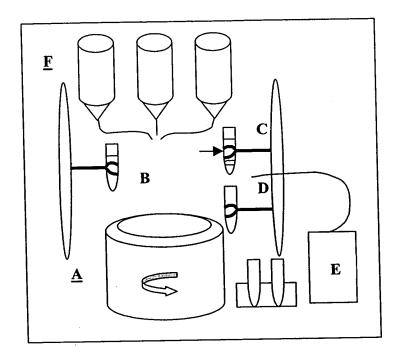
Figure 1: Novel Gene Sequence Analysis Processed cDNA Serial No. To Be Assigned sequence Docket: 506612000100 (see Example 3) Reference 1 of 10 One or more significant hits BLAST UniGene No significant hits One or more significant hits Gene Identified BLAST db_EST Cluster using No significant hits CAP2 One or more Mitochondrial gene, significant hits vector DNA, BLAST nr nonhuman gene, etc. No significant Clone remains hits unknown Unnamed genetic element **BLAST Human** No significant hits Genome One or more significant hits Download contig that contains unknown Take 100kb on Known each side of locus Protein 1. Sequence similarity to known proteins. Unknown 2. Secondary and Tertiary Protein Protein Structure Predict Analysis 3. Domain identification exons 4. Motif identification **Predict** 5. Transmembrane regions peptide 6. Antigenicity profile sequence 7. Hydropathy profile

Serial No. To Be Assigned Docket: 506612000100 Reference 2 of 10

Figure 2. Automated Mononuclear Cell RNA Isolation Device



Serial No. To Be Assigned Docket: 506612000100 Reference 3 of 10

Figure 3: Kits for discovery of, or application of diagnostic gene sets

A. Contents of kit for discovery of diagnostic gene sets

- 1. Sterile, endotoxin and RNAse free blood collection tubes (>10cc capacity)
- 2. Alcohol swabs, tourniquet, 18g needle and syringe (>10cc capacity)
- 3. Erythrocyte lysis buffer
- 4. Leukocyte lysis buffer
- 5. Substrates for labeling of RNA (may vary for various expression profiling techniques)

For fluorescence cDNA microarray expression profiling:

Reverse transcriptase and 10x RT buffer

Poly-dT primer

DTT

Deoxynucleotides 100mM each

RNAse inhibitor

Cy3 and Cy5 labeled deoxynucleotides

- 6. cDNA microarrays containing candidate gene libraries
- 7. Cover slips for slides
- 8. hybridization chambers
- 9. Software package for identification of diagnostic gene set from data

Contains statistical methods.

Allows alteration in desired sensitivity and specificity of gene set.

Software facilitates access to and data analysis by centrally located database

- 10. Password and account number to access central database server.
- 11 Kit User Manual

B. Contents of kit for application of diagnostic gene sets

- 1. Sterile, endotoxin and RNAse free blood collection tubes (>10cc capacity)
- 2. Alcohol swabs, tourniquet, 18g needle and syringe (>10cc capacity)
- 3. Erythrocyte lysis buffer
- 4. Leukocyte lysis buffer
- 5. Substrates for labeling of RNA (may vary for various expression profiling techniques)

For fluorescence cDNA microarray expression profiling:

Reverse transcriptase and 10x RT buffer

Poly-dT primer

DTT

Deoxynucleotides 100mM each

RNAse inhibitor

Cy3 and Cy5 labeled deoxynucleotides

- 6. cDNA microarrays containing diagnostic gene sets
- 7. cover slips for slides
- 8. hybridization chambers
- 9. Software package for identification of diagnostic gene set from data

Contains statistical methods.

Allows alteration in desired sensitivity and specificity of gene set.

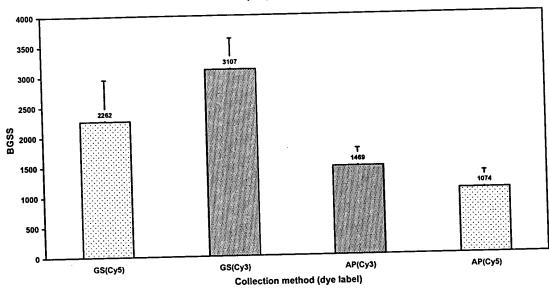
Software facilitates access to and data analysis by centrally located database server

- 10. Password and account number to access central database server.
- 11. Kit User Manual

Figure 4

Serial No. To Be Assigned Docket: 506612000100 Reference 4 of 10

Comparison of Guanine-Silica (GS) to Acid-Phenol (AP) RNA Purification



Serial No. To Be Assigned Docket: 506612000100 Reference 5 of 10

Expression of Leukocyte Specific Genes

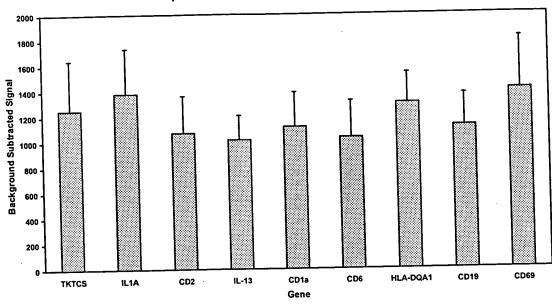
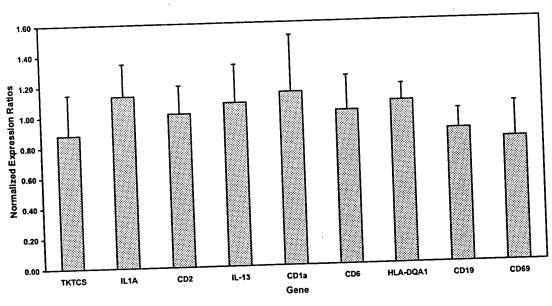


Figure 5

Figure 6

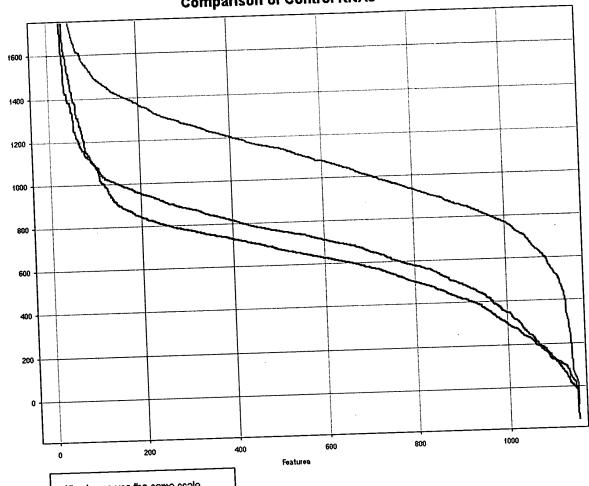
Serial No. To Be Assigned Docket: 506612000100 Reference 6 of 10

Expression of Leukocyte-Specific Genes



Serial No. To Be Assigned Docket: 506612000100 Reference 7 of 10

Comparison of Control RNAs



All columns use the same scale.

Mononuclear cells, resting and stimulated

10 Buffy Coats, resting

Mononuclear cells, resting

All markers are connected and ordered by Features.

10 µg of each control RNA was labeled.

Figure 7

Serial No. To Be Assigned Docket: 506612000100 Reference 8 of 10

Figure 8: Log expression of each probe using the R50 reference RNA. Probe expression is ordered by Signal to noise, S/N, decreasing from left to right.

Array Hybe 115018

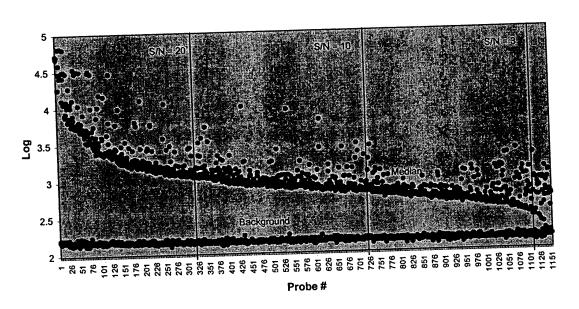
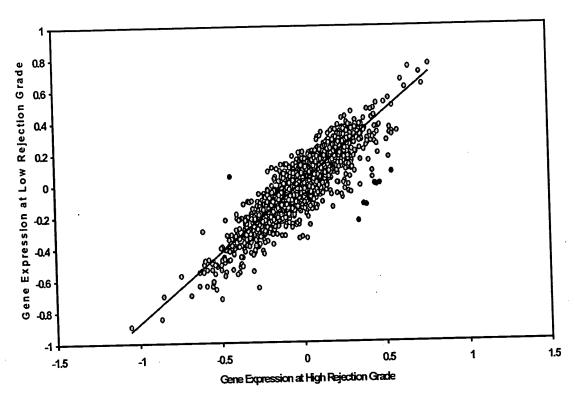


Figure 9

Serial No. To Be Assigned Docket: 506612000100 Reference 9 of 10

Comparison of High Rejection Grade to Low Rejection Grade



Serial No. To Be Assigned Docket: 506612000100 Reference 10 of 10

Figure 10: Differential gene expression between grade 0 and 3A samples:

								A	107720	· Grad	3 A	Ratio of	SRs
Probe			<u> Array 1</u>	Grade 0		Array	107739	Grau	e JA	Vallo OI			
						SR:	l _E	633	F532		SR:		
			_ 	F532 Median -	Cy3/Cy5	scale	1-		Median -	Cy3/Cy5	scaled		
		1-		B532	Ratio	ratio		3633	B532	Ratio	ratio (g/r)	Grade 0/3A	Grade 3A/U
Acc#	Name Oligo		5558	1050	0.18891			5827	358	0.061438	0.219793	3.23048873	0.3095506
M_003202	tenerintion factor 7 (T-cell specific, HMG-box) (TCF7),	2476	1810	635	0.35082	9 1.31	18579	2150	252	0.117209	0.419312	3.14462275	0.3180031
E220959	major histocompatibility complex, class II, DQ beta 1 (HL	6025	1402	487	0.34736			2121	247			3.13371968	0.3191095
E220959	major histocompatibility complex, class II, DQ beta 1 (HL	6025	804	95	0.11815			1884	75	0.039809			0.3206840
IM 002922	regulator of G-protein signalling 1 (RGS1), mRNA /cds=	2407	4121	405	0.09827			7385	254				
IM_001781	CD60 antigen (p60) early T-cell activation antigen) (CD6	2192		3447	0.2555			29882	2727	0.09125	0.326476		0.3398961
IM_002341	hypotoxin heta (TNF superfamily, member 3) (LTB), tr	2283	13488	515				1942					0.347132
E220959	major histocompatibility complex, class II, DQ beta 1 (HL	6025	1539		0.1002			7705	282	0.036	6 0.130934	2.87796556	
NM_001781	CDG0 antigen (n60 early T-cell activation antigen) (CDb	2192	3850	1119				2390	220	0.0920		2.83369583	
J05040	for unstream element (FUSE) binding protein 1 (FUBP1	3581	4507		0.12234	4 0 4	59827	9541	434	0.04548			
(14008	auclear recentor subfamily 4, group A, member 2 (NK4A	3729	1365					5310		0.06704			
NM_003202	transcription factor 7 (T-cell specific, HMG-box) (TCF7).	2476	2716					969		7 0.20330	2 0.72730		
AF035947	adoking-inducible inhibitor of signalling type 1b mRNA,	642	9850					5963	3 24	6 0.04125	4 0.14758	6 2.70062225	0.370285
NM=001781	CD69 antigen (p60, early T-cell activation antigen) (CD6	2192	. 3357	330	0.1000	71 0.0	,0007				1.1		
10_001701	X 1 1										# 7.		
											1		
				249	0.1784	17 06	370576	656	1 576	7 0.87898			
Y14737	mRNA for immunoglobulin lambda heavy chain /cds=(65	4905	1				645231	715		2 0.85375	51 3.05426		
Y14737	mPNA for immunoglobulin lambda heavy chain /cds=(65	4905	1				0.6072			8 0.84022	29 3.00588		
BC006402	mPNA for immunoglobulin lambda heavy chain /cds=(65	4481	1							0.6475			
X57812	reagranged immunoclobulin lambda light chain mRNA /c	3761	1			18 0.4	421766			6 0.6466			
X57812	rearranged immunoglobulin lambda light chain mRNA /c	3761				01 0.	520889	1732		0.8019	86 2.86907		
X72475	CDNA- EL 121321 fis clone COL02335, highly similar to	3790			-	SE 0.	51 <i>4</i> 739			5 0.8076	77 2.88943		
X72475	CDNA: FL J21321 fis. clone COL02335, highly similar to	3790	1			15 0.	489034	1		1 0.7732	99 2.76644		
X72475	CONA FI 121321 fis clone COL02335, highly similar to	3791					420551			0.6719	52 2.4038		
X57812	rearmoned immunoglobulin lambda light chain mKNA /c	3761					0.49081			34 0.8175	44 2.9247		
X72475	CDNA: FL J21321 fis. clone COL02335, highly similar to	379					0.4856			63 0.8119	36 2.9046		
X72475	CDMA: EL 121321 fis clone COL02335, highly similar to	3790					.344125	- 1		_	99 2.0605	85 0.1670035	
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	439					472005	-1			09 3.0644		
X72475	cDNA: FLJ21321 fis, clone COL02335, highly similar to	379					0.3472			69 0 639	79 2.2888	26 0.1517197	9 6.59109
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	439	- 1				.31589	-		36 0.606	62 2.1701	63 0.1455648	31 6.86979
AF967420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	439	1				.34104			37 0.6685	99 2.3918	89 0.1425836	68 7.01342
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447			34 0.09	0/4 0	,33844			75 0.6856		86 0.137979	51 7.24745
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447		-),31813			09 0.708		31 0.125598	
BC002963	coorganged immunoglobulin mRNA for mu heavy chain e	447			0.084	544 U),35593			75 0.803		45 0.123841	26 8.0748
BC002963	reagranged immunoglobulin mRNA for mu heavy chain e	447							•-		008 2.8011	84 0.123457	
BC002963	rearranged immunoclobulin mRNA for mu heavy chain e	447					34582			90 0.848		18 0.121694	77 8.21727
BC002963	rearranged immunociobulin mRNA for mu heavy chain e	447			30 0.098		0.3693				776 2.7818		63 8.260
1	rearranged immunoglobulin mRNA for mu heavy chain e	447				896 0	33675				388 2.838		
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447	75 58				0.31143				313 2.909		52 9.5537
BC002963	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	439					0.30454			597 0.888		333 0.09802	91 10.201
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	439		-			0.31176			148 0.874		181 0.087171	65 11.471
AF067420 AF067420		43	98 61	614	47 0.072	<u>2553 (</u>	0.27268	89[161	100 14	170 0.014			